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14. ABSTRACT The BCR-ABL fusion gene, the hallmark of CML, plays a causal role in the development of CML. The BCR-ABL tyrosine kinase inhibitors have been successfully used to treat patients with CML, but residual disease persists and drug resistance emerges. This clinical time bomb will have to be diffused in the not so distant future. Although BCR-ABL remains to be an attractive target for developing CML therapies, identifying and targeting additional essential components in the development of CML are important for overcoming resistance to BCR-ABL tyrosine kinase inhibitors and for eradicating leukemic cells. Substantial evidence indicates that Ras and Ras related proteins, which are commonly activated in human cancers, are critical mediators of BCR-ABL in leukemogenesis. Ras-converting enzyme (Rce1) and isoprenylcysteine carboxyl methyltransferase (Icmt) are two unique enzymes for Ras modifications that are critical for their functions. Targeted inactivation of Rce1 or Icmt is, therefore, an attractive strategy for the treatment of CML. The goal of this project is to determine whether targeted inactivation of Rce1 or Icmt could block BCR-ABL leukemogenesis. In the past funding period we have generated mice with conditional alleles of Rce1 or Icmt and used these mice to evaluate the importance of Rce1 in BCR-ABL leukemogenesis. Our preliminary results show that Rce1 plays an important role in the pathogenesis of CML.

15. SUBJECT TERMS
Chronic Myelogenous Leukemia (CML) BCR-ABL, Ras, Ras-converting enzyme (Rce1), isoprenylcysteine carboxyl methyltransferase (Icmt)

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Report

Introduction

The *BCR-ABL* fusion gene, the hallmark of CML, plays a causal role in the development of CML. The BCR-ABL tyrosine kinase inhibitors have been successfully used to treat patients with CML, but residual disease persists and drug resistance emerges. This clinical time bomb will have to be diffused in the not so distant future. Although BCR-ABL remains to be an attractive target for developing CML therapies, identifying and targeting additional essential components in the development of CML are important for overcoming resistance to BCR-ABL tyrosine kinase inhibitors and for eradicating leukemic cells. Substantial evidence indicates that Ras and Ras related proteins, which are commonly activated in human cancers, are critical mediators of BCR-ABL in leukemogenesis. Ras-converting enzyme (Rce1) and isoprenylcysteine carboxyl methyltransferase (Icmt) are two unique enzymes for Ras modifications that are critical for their functions. Targeted inactivation of Rce1 or Icmt is, therefore, an attractive strategy for the treatment of CML. The goal of this project is to determine whether targeted inactivation of Rce1 or Icmt could block BCR-ABL leukemogenesis. In the past funding period we have generated mice with conditional alleles of Rce1 or Icmt and used these mice to evaluate the importance of Rce1 and Icmt in BCR-ABL leukemogenesis. Our preliminary results show that Icmt plays an important role in the pathogenesis of CML.

Body

1. (Task 1) We have generated $Rce1^{flx/flx}$ or $Icmt^{flx/flx}$ mice, as well as $Rce1^{flx/+}$, $Icmt^{flx/+}$, $Rce1^{+/+}$, or $Icmt^{+/+}$ control mice harboring the Mx1-Cre transgene through breeding $Rce1^{flx/+}$/Mx1-Cre and $Icmt^{flx/+}$/Mx1-Cre mice.

2. (Task 6) Since last submission, we have made new progress on the project, which is reported here. We examined the leukemogenic potential of BCR-ABL with or without Rce1 or Icmt using the mouse bone marrow transduction and transplantation model for CML as proposed. Briefly Cre expression was induced in $Rce1^{flx/flx}$/Mx1-Cre, $Rce1^{flx/+}$/Mx1-Cre, $Icmt^{flx/flx}$/Mx1-Cre, $Icmt^{flx/+}$/Mx1-Cre, or Mx1-Cre control mice by intraperitonieal injections of polyinosinic-polycytidylic ribonucleic acid (pI-pC) for 4 times. These mice were then treated with 5-fluoruracil (5-FU) for 4 days to enrich and activate hematopoietic stem cells. Bone marrow (BM) cells from the above mice were isolated and transduced with *BCR-ABL* retroviruses. As shown in Figure 1, BCR/ABL leukemogenic potential was reduced in pI-pC-induced $Rce1^{flx/+}$/Mx1-Cre mice, compared to BCR/ABL transduced $Rce1^{+/+}$/Mx1-Cre mice. However, BCR/ABL transduced $Rce1^{flx/flx}$/Mx1-Cre mice developed disease much faster then the control mice with wt Rce1. These data suggest that the role of Rce1 in BCR/ABL leukemogenesis is complicated and dependent on the dosage. This may due to the fact that there are many substrates of Rce1, some of them may facilitate BCR-ABL leukemogenesis, while others may inhibit. We will repeat this experiment and confirm the finding.

Unlike complicated result of Rce1 inactivation, Icmt inactivation significantly mitigated BCR-ABL leukemogenesis (Figure 2). This result suggests that Icmt plays an critical role in BCR-ABL leukemogenesis and that Icmt may be an effective target for developing CML therapeutics.

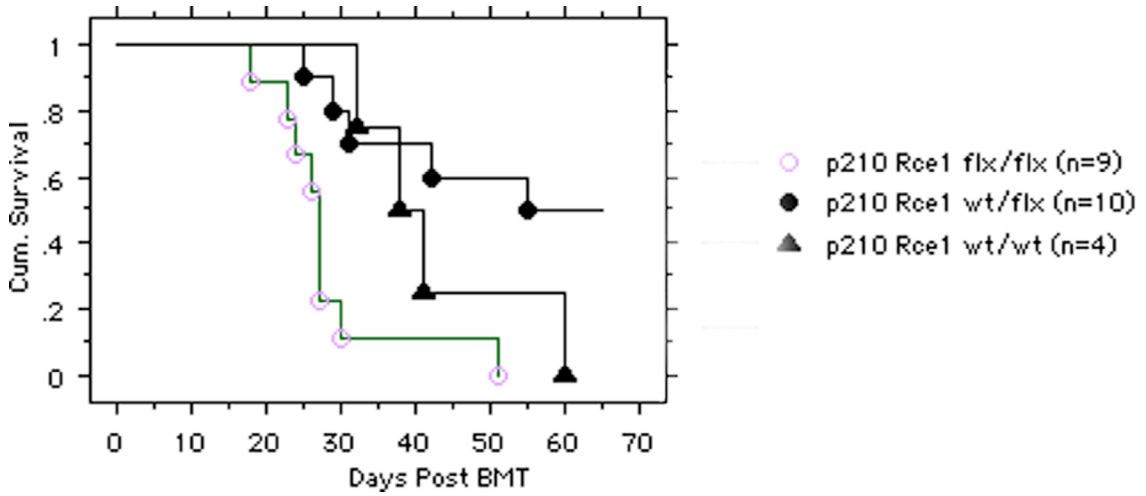

Figure 1. Survival of mice receiving transplantation of *BCR-ABL* transduced BM cells from pI-pC-induced $Rce1^{flx/flx}$/Mx1-Cre, $Rce1^{flx/+}$/Mx1-Cre, $Rce1^{+/+}$/Mx1-Cre mice. Survival curves were generated by Kaplan-Meier survival analysis. The number (n) of mice for each group is indicated.

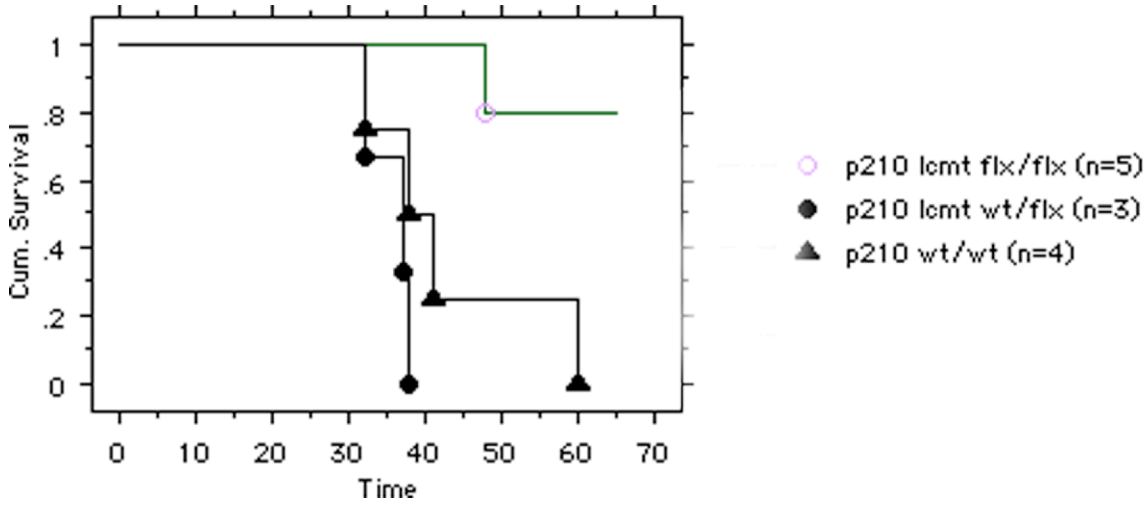

Figure 2. Survival of mice receiving transplantation of *BCR/ABL* transduced BM cells from pI-pC-induced $Icmt^{flx/flx}$/Mx1-Cre, $Icmt^{flx/+}$/Mx1-Cre, $Icmt^{+/+}$/Mx1-Cre mice. Survival curves were generated by Kaplan-Meier survival analysis. The unit of time is days post-BM transplantation. The number (n) of mice for each group is indicated.

Since Icmt and Rce1 are important for BCR-ABL leukemogenesis, we will generate MCSV-*BCR-ABL*/gfp-IRES-*Icmt* and MCSV-*BCR-ABL*/gfp-IRES-*Rce1* bicistronic retroviral constructs (Task 2) to examine whether the transforming potential of

BCR-ABL could be rescued by coexpressing Icmt or Rce1 (Task 4). We are currently performing the in vitro BCR-ABL transformation assay (factor independent bone marrow colony assay) as planed in Task 3.

3. (Related to Tasks 5 and 9). In addition to Ras proteins, a broad class of proteins, many of which are also involved in cellular regulatory processes that are important for tumor formation, are also substrates of Rce1 and Icmt. Rce1 and Icmt may affect BCR-ABL leukemogenesis through Ras proteins and/or other targets of the enzymes. Myritoylated Ras would help to evaluate whether the effect Rce1 deficiency on BCR-ABL leukemogenesis is primarily through Ras, but it does not directly testing the Rce1 and Icmt mediated Ras modifications. We have previously found that the Grb2 SH2 binding site Y177 of BCR-ABL, the major mediator of BCR-ABL inactivating Ras, is required for the induction of CML-like disease by BCR-ABL. Recently we further show that oncogenic Ras can rescue the defect of the Y177F mutant BCR-ABL in the induction of CML-like disease. We will use this system to examine the importance of Ras modifications in BCR-ABL leukogenesis. To this end, we have generated an A2 (the second alphatic residue in the CAAX motif) mutant of NRASD12 for testing the importance of removal of the AAX tripeptide and methylation of the terminal farnesylated cysteine residue in a different way (this would avoid complications of targeting proteins other than RAS). The A2 residue has been shown to be important for the AAX peptide cleavage and methylation.

Key research accomplishment

- We have generated $Rce1^{flx/flx}$ or $Icmt^{flx/flx}$ mice, as well as $Rce1^{flx/+}$, $Icmt^{flx/+}$, $Rce1^{+/+}$, or $Icmt^{+/+}$ control mice harboring the Mx1-Cre transgene.
- We have found that Icmt plays a critical role in BCR-ABL leukemogenesis. This finding suggests that Icmt may be an effective target for developing CML therapeutics.
- We have found that the role of Rce1 in BCR-ABL leukemogenesis is dosage dependent.

Reportable outcomes
Not yet.

Conclusion

Our results show that Icmt plays an important role in the pathogenesis of CML and suggest that this enzyme is an effective target for developing CML therapies. Our results also show that the role of Rce1 in BCR-ABL leukemogenesis is dosage dependent.

References
NA

Appendices
NA